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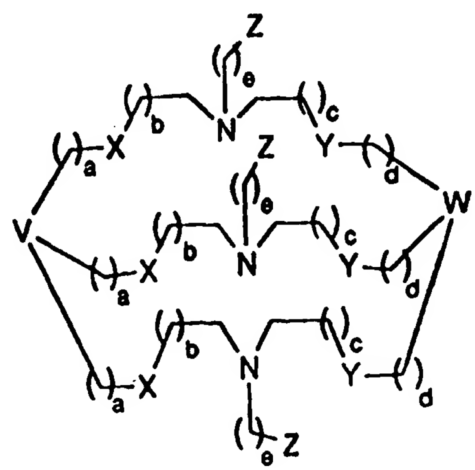
(54) Title: FUNCTIONALIZED AZA-CRYPTAND LIGANDS FOR IMAGING APPLICATIONS

(57) Abstract

The present invention provides new and structurally diverse compositions comprising compounds of general formula (I), wherein V is CR_1 or N; W is CR_2 or N; R_1 and R_2 may be the same or different and are hydrogen, C_1-C_8alkyl, or C_6-C_{10}aryl, optionally R_1 and R_2 may be substituted by one or more hydroxy, C_1-C_8alkyl, C_1-C_8hydroxyalkyl, C_1-C_8alkoxy, C_6-C_{10}aryl, C_6-C_{10}hydroxyaryl, C_6-C_{10}aryloxy, $-CO_2R_3$, $CONR_4R_5$, or $-NR_4R_5$; X and Y may be the same or different and are O, NR_6, S, or CR_7R_8; R_3, R_4, R_5, R_6, R_7 and R_8 may be the same or different and are hydrogen, C_1-C_8alkyl, C_1-C_8hydroxyalkyl, or C_1-C_8alkoxyalkyl; R_4 and R_5 may form a 5 or 6 membered carbocyclic ring optionally containing singularly or in combination nitrogen, oxygen or sulfur; a, b, c, d and e may be the same or different and are zero to about 10; and Z is $-CO_2H$, $-PO_3H_2$, $-SO_3H$, or -CONHOH. Methods for imaging using compositions of the invention are also provided.



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FUNCTIONALIZED AZA-CRYPTAND LIGANDS FOR IMAGING APPLICATIONS

FIELD OF THE INVENTION

This invention relates to magnetic resonance imaging (MRI), x-ray imaging, and radiopharmaceuticals. More particularly the invention relates to methods and compositions for enhancing MRI, x-ray imaging, and radiopharmaceuticals.

BACKGROUND OF THE INVENTION

The use of contrast agents in diagnostic medicine is rapidly growing. In X-ray diagnostics, for example, increased contrast of internal organs, such as the kidneys, the urinary tract, the digestive tract, the vascular system of the heart (angiography), and so forth is obtained by administering a contrast agent which is substantially radiopaque. In conventional proton MRI diagnostics, increased contrast of internal organs and tissues may be obtained by administering compositions containing paramagnetic metal species which increase the relaxivity of surrounding protons. In ultrasound diagnostics, improved contrast is obtained by administering compositions having acoustic impedances different than that of blood and other tissues.

The recently developed technique of MRI encompasses the detection of certain atomic nuclei utilizing magnetic fields and radio-frequency radiation. It is similar in some respects to X-ray computed tomography (CT) in providing a cross-sectional display of the body organ anatomy with

excellent resolution of soft tissue detail. As currently used, the images produced constitute a map of the proton density distribution ,the relaxation times, or both, in organs and tissues. The technique of MRI is advantageously non-invasive as it avoids the use of ionizing radiation.

While the phenomenon of NMR was discovered in 1945, it is only recently that it has found application as a means of mapping the internal structure of the body as a result of the original suggestion of Lauterbur (Nature, 242, 190-191 [1973]). The fundamental lack of any known hazard associated with the level of the magnetic and radio-frequency fields that are employed renders it possible to make repeated scans on vulnerable individuals. In addition to standard scan planes (axial, coronal, and sagittal), oblique scan planes can also be selected.

With an MRI experiment, the nuclei under study in a sample (e.g. protons) are irradiated with the appropriate radio-frequency (RF) energy in a highly uniform magnetic field. These nuclei, as they relax, subsequently emit RF at a sharp resonance frequency. The resonance frequency of the nuclei depends on the applied magnetic field.

According to known principles, nuclei with appropriate spin, when placed in an applied magnetic field (B, expressed generally in units of gauss or Tesla [10^4 gauss]) align in the direction of the field. In the case of protons, these nuclei precess at a frequency, f, of 42.6 MHz, at a field strength of 1 Tesla. At this frequency, an RF pulse of radiation will excite the nuclei and can be considered to tip the net magnetization out of the field direction, the extent of this rotation being determined by the pulse duration and energy. After the RF pulse, the nuclei "relax" or return to equilibrium with the magnetic field, emitting radiation at the resonant frequency. The decay of the emitted radiation is

characterized by two relaxation times, i.e., T_1, the spin-lattice relaxation time or longitudinal relaxation time, that is, the time taken by the nuclei to return to equilibrium along the direction of the externally applied magnetic field, and T_2, the spin-spin relaxation time associated with the dephasing of the initially coherent precession of individual proton spins. These relaxation times have been established for various fluids, organs and tissues in different species of mammals.

In MRI, scanning planes and slice thicknesses can be selected. This selection permits high quality transverse, coronal and sagittal images to be obtained directly. The absence of any moving parts in MRI equipment promotes high reliability. It is believed that MRI has a greater potential than CT for the selective examination of tissue characteristics in view of the fact that in CT, X-ray attenuation coefficients alone determine image contrast, whereas at least five separate variables (T_1, T_2, proton density, pulse sequence and flow) may contribute to the MRI signal.

By reason of its sensitivity to subtle physico-chemical differences between organs and/or tissues, it is believed that MRI may be capable of differentiating different tissue types and in detecting diseases which induce physicochemical changes that may not be detected by X-ray or CT which are only sensitive to differences in the electron density of tissue.

As noted above, two of the principal imaging parameters are the relaxation times, T_1 and T_2. For protons (or other appropriate nuclei), these relaxation times are influenced by the environment of the nuclei, (e.g., viscosity, temperature, and the like). These two relaxation

phenomena are essentially mechanisms whereby the initially imparted radio-frequency energy is dissipated to the surrounding environment. The rate of this energy loss or relaxation can be influenced by certain other nuclei which are paramagnetic. Chemical compounds incorporating these paramagnetic nuclei may substantially alter the T_1 and T_2 values for nearby protons. The extent of the paramagnetic effect of a given chemical compound is a function of the environment.

In general, paramagnetic species such as ions of elements with atomic numbers of 21 to 29, 42 to 44 and 58 to 70 have been found effective as MRI image contrasting agents. Examples of suitable ions include chromium(III), manganese(II), manganese(III), iron(II), iron(III), cobalt(II), nickel(II), copper(II), praseodymium(III), neodymium(III), samarium(III), and ytterbium(III). Because of their very strong magnetic moments, gadolinium(III), terbium(III), dysprosium(III), holmium(III) and erbium(III) are preferred. Gadolinium(III) ions have been particularly preferred as MRI contrasting agents.

Typically, paramagnetic ions have been administered in the form of complexes with organic complexing agents. Such complexes provide the paramagnetic ions in a soluble, non-toxic form, and facilitate their rapid clearence from the body following the imaging procedure. Gries et al., U.S. Patent 4,647,447, disclose complexes of various paramagnetic ions with conventional aminocarboxylic acid complexing agents. A preferred complex disclosed by Gries et al. is the complex of gadolinium(III) with diethylenetriamine-pentaacetic acid ("DTPA").

Paramagnetic ions, such as gadolinium(III), have been found to form strong complexes with DTPA, ethylenediamine-tetraacetic acid ("EDTA"), and with tetraazacyclododecane-N,N',N",N"'-tetraacetic acid ("DOTA").

These complexes do not dissociate substantially in physiological aqueous fluids. The gadolinium complex of DTPA has a net charge of -2, whereas the gadolinium complex of EDTA or DOTA has a net charge of -1, and both are generally administered as soluble salts. Typical salts are sodium and N-methylglucamine. The administration of salts is attended by certain disadvantages. These salts can raise the in vivo ion concentration and cause localized disturbances in osmolality, which in turn, can lead to edema and other undesirable reactions.

Efforts have been made to design new ionic and neutral paramagnetic metal complexes which avoid or minimize the above mentioned disadvantages. In general, this goal can be achieved by converting one or more of the free carboxylic acid groups of the complexing agent to neutral, non-ionizable groups. For example, S.C. Quay, in U.S. Patents 4,687,658 and 4,687,659, discloses alkylester and alkylamide derivatives, respectively, of DTPA complexes. Similarly, published Dean et al., U.S. Patent Number 4,826,673 discloses mono- and polyhydroxyalkylamide derivatives of DTPA and their use as complexing agents for paramagnetic ions. It can also be achieved by covalent attachment of organic cations to the complexing agent in such a manner that the sum of positive and negative charges in the resulting metal complex is zero.

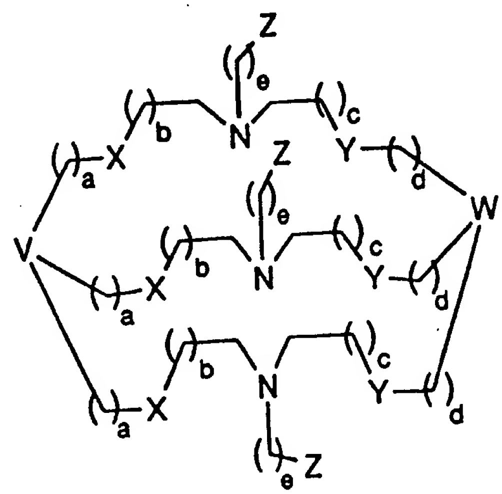
The nature of additional substituents in the complexing agent can have a significant impact on tissue specificity. Hydrophilic complexes tend to concentrate in the interstitial fluids, whereas lipophilic complexes tend to associate with cells. Thus, differences in hydrophilicity can lead to different applications of the compounds. See, for example, Weinmann et al., AJR, 142, 679 (Mar. 1984) and Brasch, et al., AJR, 142, 625 (Mar. 1984).

Finally, toxicity of paramagnetic metal complexes is greatly affected by the nature of the complexing agents. In vivo release of free metal ions from the complex is a major cause of toxicity. Four principal factors are important in the design of chelates for making paramagnetic metal complexes that are highly stable *in vivo* and less toxic. The first three factors are thermodynamic in nature whereas the fourth involves chelate kinetics. The first factor is the thermodynamic stability constant of the metal-ligand. The thermodynamic stability constant indicates the affinity that the totally unprotonated ligand has for a metal. The second factor is the conditional stability constant which takes into account the pH and is important when considering stability under physiological pH. The selectivity of the ligand for the paramagnetic metal over other endogenous metal ions such as zinc, iron, magnesium and calcium is the third factor. In addition to the three thermodynamic considerations, complexes with structural features that make in vivo transmetallation reactions much slower than their clearance rates would be predicted to have low toxicities. Therefore, in vivo reaction kinetics are a major factor in the design of stable complexes. See, for example, Cacheris et al., Magnetic Resonance Imaging, 8:467 (1990) and Oksendal, et al., JMRI, 3:157 (1993).

A need continues to exist for new and structurally diverse compounds for use as imaging agents and radiopharmaceuticals. There is a further need to develop highly stable complexes with good relaxivity and osmolar characteristics.

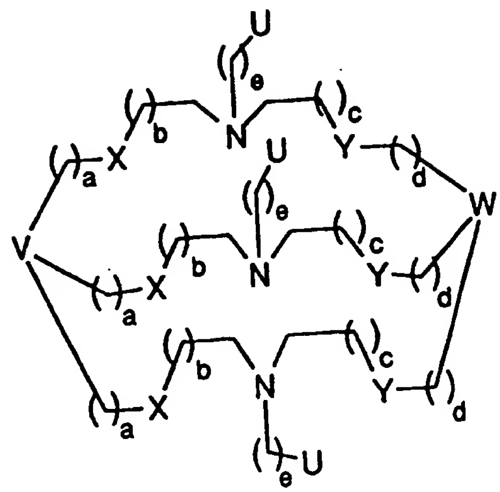
SUMMARY OF THE INVENTION

The present invention provides new and structurally diverse compositions comprising compounds of the general formula:



Wherein V is CR_1 or N; W is CR_2 or N; R_1 and R_2 may be the same or different and are hydrogen, C_1-C_8 alkyl, or C_6-C_{10} aryl, optionally R_1 and R_2 may be substituted by one or more hydroxy, C_1-C_8 alkyl, C_1-C_8 hydroxyalkyl, C_1-C_8 alkoxy, C_6-C_{10} aryl, C_6-C_{10} hydroxyaryl, C_6-C_{10} aryloxy, $-CO_2R_3$, $CONR_4R_5$, or $-NR_4R_5$; X and Y may be the same or different and are O, NR_6, S, or CR_7R_8; R_3, R_4, R_5, R_6, R_7 and R_8 may be the same or different and are hydrogen, C_1-C_8 alkyl, C_1-C_8 hydroxyalkyl, or C_1-C_8 alkoxyalkyl; R_4 and R_5 may form a 5 or 6 membered carbocyclic ring optionally containing singularly or in combination nitrogen, oxygen or sulfur; a, b, c, d and e may be the same or different and are zero to about 10; and Z is $-CO_2H$, $-PO_3H_2$, $-SO_3H$, -CONHOH.

Also provided are compositions comprising complexes of the compounds with metal ions of the general formula:



Wherein V is CR_1 or N; W is CR_2 or N; R_1 and R_2 may be the same or different and are hydrogen, C_1-C_8 alkyl, or C_6-C_{10} aryl, optionally R_1 and R_2 may be substituted by one or more hydroxy, C_1-C_8 alkyl, C_1-C_8 hydroxyalkyl, C_1-C_8 alkoxy, C_6-C_{10} aryl, C_6-C_{10} hydroxyaryl, C_6-C_{10} aryloxy, $-CO_2R_3$, $CONR_4R_5$, or $-NR_4R_5$; X and Y may be the same or different and are O, NR_6, S, or CR_7R_8. R_3, R_4, R_5, R_6, R_7 and R_8 may be the same or different and are hydrogen, C_1-C_8 alkyl, C_1-C_8 hydroxyalkyl or C_1-C_8 alkoxyalkyl; R_4 and R_5 may form a 5 or 6 membered carbocyclic ring optionally containing singularly or in combination nitrogen, oxygen or sulur; a, b, c, d and e may be the same or different and are zero to about 10; U is $-CO_2M$, $-PO_3HM$, $-SO_3M$ or CONHOM; and M is a metal ion or equivalent and/or a physiologically acceptable cation of an inorganic or organic base.

Compositions comprising the above formulas wherein M is a radioactive metal ion, a paramagnetic ion, or a metal ion capable of absorbing x-rays are also provided for use as radiopharmaceuticals, magnetic resonance imaging, and x-ray contrast agents, respectively.

Diagnostic compositions comprising the compounds of the

invention are also provided. Methods of performing diagnostic procedures with compositions of the invention are also disclosed. The methods comprise administering to a patient an effective amount of the compositions of the invention and optionally subjecting the patient to an imaging procedure of imaging.

DETAILED DESCRIPTION

The compositions of the invention are suitable for use with a variety of modalities including x-rays, magnetic resonance imaging and radiopharmaceuticals.

The functionality of the R groups of the compositions of the invention afford the additional capability of derivatization to biomolecules and synthetic polymers. Biomolecule refers to all natural and synthetic molecules that play a role in biological systems. Biomolecules include hormones, amino acids, peptides, peptidomimetics, proteins, deoxyribonucleic acid (DNA) ribonucleic acid (RNA), lipids, albumins, polyclonal antibodies, receptor molecules, receptor binding molecules, monoclonal antibodies and aptamers. Specific examples of biomolecules include insulins, prostaglandins, growth factors, liposomes and nucleic acid probes. Examples of synthetic polymers include polylysine, arborols, dendrimers, and cyclodextrins. The advantages of using biomolecules include enhanced tissue targeting through specificity and delivery. Coupling of the chelating moieties to biomolecules can be accomplished by several known methods (e.g., Krejcarek and Tucker Biochem. Biophys. Res. Comm, **30**, 581 (1977); Hnatowich, et al. Science, **220**, 613 (1983). For example, a reactive moiety present in one of the R groups is coupled with a second reactive group located on the biomolecule. Typically, a nucleophilic group is reacted with an electrophilic group to form a covalent bond between the biomolecule and the chelate. Examples of nucleophilic groups

include amines, anilines, alcohols, phenols, thiols and hydrazines. Electrophilic group examples include halides, disulfides, epoxides, maleimides, acid chlorides, anhydrides, mixed anhydrides, activated esters, imidates, isocyanates and isothiocyanates. And finally, the compositions of the invention should provide the additional advantage of being kinetically inert.

Examples of suitable alkyl groups for use with the invention include methyl, ethyl, propyl, isopropyl, butyl, cyclohexyl, heptyl and octyl. Suitable alkoxy groups include methoxy, ethoxy, propoxy, butoxy, pentoxy, hexoxy, heptoxy and octoxy. Hydroxyalkyl groups suitable for use with the invention include both mono and poly hydroxyalkyls such as hydroxyethyl, 2-hydroxypropyl, 2,3-dihydroxypropyl, 2,3,4-trihydroxybutyl, tris(hydroxymethyl)methyl and 2-hydroxy-1-hydroxymethyl-ethyl. Suitable alkoxyalkyl groups include methoxymethyl, 2,3-dimethoxypropyl, tris (methoxymethyl)methyl, and 2-methoxy-1-methoxymethyl-ethyl.

Examples of suitable compounds of the invention are 1, 11-dimethyl-3, 9, 13, 19, 22, 28-hexaoxa-(N^6, N^{16}, N^{25}-tricarboxymethyl)-6, 16, 25-triaza-bicyclo [9.9.9] nonacosane; 1-hydroxymethyl-13-(2-aminoethyl)-(N^3, N^{11}, N^{15}, N^{23}, N^{26}, N^{34}-hexamethyl)-(N^7, N^{19}, N^{30}-tricarboxymethyl)-3, 7, 11, 15, 19, 23, 26, 30, 34-nonaza-bicyclo [11.11.11]-penta triacontane HSA conjugate; 4, 10, 16, 22, 27, 33-hexaoxa-[N^7, N^{19}, N^{30}-(hydroxylamino) carboxymethyl]-1, 7, 13, 19, 30-pentaaza-bicyclo [11.11.11]-pentatriacontane; and 1-hydroxymethyl-11-carboxy-3, 9, 13, 19, 22, 28-hexathio-(N^6, N^{16}, N^{25}-trisulfomethyl)-6, 16, 25-triaza-bicyclo [9.9.9] nonacosane poly-L-lysine conjugate.

Complexes of the novel ligands or compounds of the invention with one or more central metal ions or metal ion equivalents such as paramagnetic metals praseodymium(III),

neodymium(III), samarium(III), ytterbium(III) terbium(III), dysprosium(III), holmium(III), erbium(III), iron(II), iron(III), manganese(II), manganese(III), gadolinium(III), chromium(III), cobalt(II) and nickel(II) are useful for enhancing magnetic resonance images. While such metal ions are themselves paramagnetic in nature and capable of altering the magnetic resonance signal characteristics of body tissues, organs or fluids, they may exhibit significant toxicity when administered in the form of ionic salts. However, novel complexes of the invention are relatively or substantially nontoxic and therefore useful for enhancing magnetic resonance images by favorably altering relaxation times T_1 and T_2 and affording improved contrast between normal and diseased tissues or organs.

The preferred complexes of the invention are those formed from the above ligands and iron(II), iron(III), manganese(II), manganese(III) and gadolinium(III) as the central metal ion or ions. Depending upon the particular ligand employed and the particular central metal ion used, the complexes formed may be neutral, ionic, cationic, or zwitterionic in nature, or they may be negatively charged. The neutral complexes are generally preferred and generally appear to exhibit relatively lower toxicity as compared to ionic or negatively charged complexes. The negatively charged complexes formed by the ligands and central metal ions enumerated above may be further complexed with one or more cations of an inorganic or organic base which are physiologically tolerated. Examples of cations for further complexing include sodium, potassium, calcium, and salts of N-methylglucamine, and diethanolamine.

Examples of preferred compounds of the invention and one or more central metal ions (i.e., complexes) include

1,11-dimethyl-3,9,13,19,22,28-hexaoxa-(N^6, N^{16}, N^{25}-tricarboxymethyl)-6,16,25-triaza-bicyclo[9.9.9]nonacosane, gadolinium(III) complex,

1-hydroxymethyl-13-(2-aminoethyl)-(N^3, N^{11}, N^{15}, N^{23}, N^{26}, N^{34}-hexamethyl)-(N^7, N^{19}, N^{30}-tricarboxymethyl)-3,7,11,15,19,23,26,30,34-nonaaza-bicyclo[11.11.11]-pentatriacontane, HSA conjugate, dysprosium(III) complex,

1-hydroxymethyl-13-(2-aminoethyl)-(N^3, N^{11}, N^{15}, N^{23}, N^{26}, N^{34}-hexamethyl)-(N^7, N^{19}, N^{30}-tricarboxymethyl)-3,7,11,15,19,23,26,30,34-nonaaza-bicyclo[11.11.11]-pentatriacontane, HSA conjugate, ytterbium(III) complex,

4,10,16,22,27,33-hexaoxa-[N^7, N^{19}, N^{30}-(hydroxylamino)carboxymethyl]-1,7,13,19,30-pentaaza-bicyclo[11.11.11]-pentatriacontane, iron(III) complex,

4,10,16,22,27,33-hexaoxa-[N^7, N^{19}, N^{30}-(hydroxylamino)carboxymethyl]-1,7,13,19,30-pentaaza-bicyclo[11.11.11]-pentatriacontane, ytterbium(III) complex, and

1-hydroxymethyl-11-carboxy-3,9,13,19,22,28-hexathio-(N^6, N^{16}, N^{25}-trisulfomethyl)-6,16,25-triaza-bicyclo[9.9.9]nonacosane, poly-L-lysine conjugate, gadolinium(III) complex.

In addition to their utility in magnetic resonance imaging procedures, the compositions of the invention can also be employed for delivery of either radiopharmaceuticals or heavy metals for x-ray contrast into the body. For use in diagnostic and therapeutic radiopharmaceuticals the complexed metal ion must be radioactive. Radioisotopes of the elements technetium, rhenium, indium, gallium, copper, yttrium, samarium and holmium are suitable. For use as X-ray contrast applications the complexed metal ion must be able to absorb adequate amounts of the X-rays. These metal ions are generally refered to as radioopaque. Suitable elements for use as the radioopaque

metal ion include lead, bismuth, gadolinium, dysprosium, holmium and praseodymium.

Examples of preferred compounds for radiopharmaceuticals are 1,11-dimethyl-3,9,13,19,22,28-hexaoxa-(N^6, N^{16}, N^{25}-tricarboxymethyl)-6,16,25-triaza-bicyclo[9.9.9]nonacosane, gallium(III) complex,

1,11-dimethyl-3,9,13,19,22,28-hexaoxa-(N^6, N^{16}, N^{25}-tricarboxymethyl)-6,16,25-triaza-bicyclo[9.9.9]nonacosane, yttrium(III) complex,

1-hydroxymethyl-13-(2-aminoethyl)-(N^3, N^{11}, N^{15}, N^{23}, N^{26}, N^{34}-hexamethyl)-(N^7, N^{19}, N^{30}-tricarboxymethyl)-3,7,11,15,19,23,26,30,34-nonaaza-bicyclo[11.11.11]-pentatriacontane, HSA conjugate, indium(III) complex,

1-hydroxymethyl-11-carboxy-3,9,13,19,22,28-hexathio-(N^6, N^{16}, N^{25}-trisulfomethyl)-6,16,25-triaza-bicyclo[9.9.9]nonacosane, poly-L-lysine conjugate, technetium(V) complex, and

1-hydroxymethyl-11-carboxy-3,9,13,19,22,28-hexathio-(N^6, N^{16}, N^{25}-trisulfomethyl)-6,16,25-triaza-bicyclo[9.9.9]nonacosane, poly-L-lysine conjugate, rhenium(V) complex.

Examples of preferred compounds for x-ray contrast are 1,11-dimethyl-3,9,13,19,22,28-hexaoxa-(N^6, N^{16}, N^{25}-tricarboxymethyl)-6,16,25-triaza-bicyclo[9.9.9]nonacosane, bismuth(III) complex,

1-hydroxymethyl-13-(2-aminoethyl)-(N^3, N^{11}, N^{15}, N^{23}, N^{26}, N^{34}-hexamethyl)-(N^7, N^{19}, N^{30}-tricarboxymethyl)-3,7,11,15,19,23,26,30,34-nonaaza-bicyclo[11.11.11]-pentatriacontane, HSA conjugate, holmium(III) complex,

4,10,16,22,27,33-hexaoxa-[N^7,N^{19}, N^{30}-(hydroxylamino)carboxymethyl]-1,7,13,19,20-

pentaaza-bicyclo[11.11.11]-pentatriacontane, dysprosium(III) complex,

4,10,16,22,27,33-hexaoxa-[N^7,N^{19}, N^{30}-(hydroxylamino)carboxymethyl]-1,7,13,19,20-pentaaza-bicyclo[11.11.11]-pentatriacontane, bismuth(III) complex, and

1-hydroxymethyl-11-carboxy-3,9,13,19,22,28-hexathio-(N^6, N^{16}, N^{25}-trisulfomethyl)-6,16,25-triaza-bicyclo[9.9.9]nonacosane, poly-L-lysine conjugate, dysprosium(III) complex..

The compositions of the invention can be formulated into therapeutic or diagnostic compositions for enteral or parenteral administration. These compositions contain an effective amount of the paramagnetic ion complex along with conventional pharmaceutical carriers and excipients appropriate for the type of administration contemplated. For example, parenteral formulations advantageously contain a sterile aqueous solution or suspension of from about 0.05 to about 1.0M of a paramagnetic ion complex according to this invention. Parenteral compositions may be injected directly or mixed with a large volume parenteral composition for systemic administration. Preferred parenteral formulations have a concentration of paramagnetic ion complex of about 0.1M to about 0.5M. Such solutions also may contain pharmaceutically acceptable buffers and, optionally, electrolytes such as sodium chloride. The compositions may advantageously contain a slight excess (e.g., from about 0.01 to about 15.0 mole % excess) of a complexing agent or its complex with a physiologically acceptable, non-toxic cation. Such physiologically acceptable, non-toxic cations include calcium ions, magnesium ions, copper ions, zinc ions, salts of n-methylglucamine and diethanolamine, and the like. Generally, calcium ions are preferred.

Formulations for enteral administration may vary widely, as is well-known in the art. In general, such formulations are liquids which include an effective amount of the paramagnetic ion complex in aqueous solution or suspension. Such enteral compositions

may optionally include buffers, surfactants, thixotropic agents, and the like. Compositions for oral administration may also contain flavoring agents and other ingredients for enhancing their organoleptic qualities.

The diagnostic compositions are administered in doses effective to achieve the desired enhancement of the image. Such doses may vary widely, depending upon the particular paramagnetic ion complex employed, the organs or tissues which are the subject of the imaging procedure, the imaging procedure, the imaging equipment being used, and the like. In general, parenteral dosages will range from about 0.001 to about 1.0 mMol of paramagnetic ion complex per kg of patient body weight. Preferred parenteral dosages range from about 0.01 to about 0.5mMol of paramagnetic ion complex per kg of patient body weight. Enteral dosages generally range from about 0.5 to about 100 mMol, preferably from about 1.0 to about 10 mMol, preferably from about 1.0 to about 20.0 mMol of paramagnetic ion complex per kg of patient body weight.

The diagnostic compositions of the invention are used in the conventional manner. The compositions may be administered to a patient, typically a warm-blooded animal, either systemically or locally to the organ or tissue to be imaged, and the patient then subjected to the imaging procedure. Protocols for imaging and instrument procedures are found in texts such as Stark, D.D.; Bradley, W.G. *Magnetic Resonance Imaging*; Mosby Year Book: St. Louis, MO, 1992.

Radiopharmaceutical Imaging Procedures are found in Fred A. Mettler, Jr., M.D., M.P.H., Milton J. Guiberteau, M.D., <u>Essentials of Nuclear Medicine Imaging</u>, Grune and Stratton, Inc., New York, NY 1983) and E. Edmund Kim, M.S., M.D. and Thomas P. Haynie, M.D., (MacMillan Publishing Co. Inc., New York, NY 1987).

X-ray contrast Imaging Procedures are found in Albert A. Moss, M.D., Gordon Gamsu, M.D., and Harry K. Genant, M.D., <u>Computed Tomography of the Body</u>, (W.B. Saunders Company, Philadelphia, Pennsylvania 1992) and M. Sovak, Editor,

Radiocontrast Agents, (Springer-Verlag, Berlin 1984).

The following examples illustrate the specific embodiments of the invention described in this document. As would be apparent to skilled artisans, various changes and modifications are possible and are contemplated within the scope of the invention described.

EXAMPLES

Example 1

Synthesis of 1, 11-dimethyl-3, 9, 13, 19, 22, 28-hexaoxa-(N^6, N^{16}, N^{25}-tricarboxymethyl)-6, 16, 25-triaza bicyclo[9.9.9]nonacosane

A solution of 2,2-dimethyl-5-hydroxymethyl-5-methyl-1,3-dioxane (13.4 g, 0.084 mol) in 630 mL of anhydrous tetrahydrofuran was cooled to -20C under argon atmosphere. Then a 0.5 M solution of potassium bis(trimethylsilyl)amide in toluene (168 mL, 0.084 mol) was added and the mixture was stirred at -20C for 1 hr. The 2-benzyloxyethyltriflate (23.8 g, 0.084 mol) was added neat, keeping the temperature between -20 and -15C. The resulting solution was stirred at this temperature for 1 hr, then gradually allowed to warm to 25C and stirred for 15 hrs. Dichloromethane (900 mL) was added and the solution was washed twice with 300 mL of water and once with 100 mL of saturated sodium chloride solution. The organic layer was dried over anhydrous sodium sulfate and evaporation of the solvents under reduced pressure gave 2,2-dimethyl-5-benzyloxyethoxymethyl-5-methyl-1,3-dioxane (24.6 g, 0.084 mol, 100%) which was not purified.

A solution of 2,2-dimethyl-5-benzyloxyethoxymethyl-5-methyl-1,3-dioxane (24.6 g, 0.084 mol) in 200 mL of 10% hydrochloric acid and 200 mL of tetrahydrofuran was stirred at 25C for 4 hrs. The pH of the mixture was adjusted to 7 with solid sodium

bicarbonate and the aqueous solution was extracted several times with dichloromethane. The combined organic extracts were dried over anhydrous sodium sulfate and evaporated under reduced pressure to give 1-benzyloxyethoxymethyl-1,1-dihydroxymethylethane (18.4 g, 0.072 mol, 86%).

A solution of 1-benzyloxyethoxymethyl-1,1-dihydroxymethylethane (12.9 g, 0.051 mol) in 600 mL of anhydrous tetrahydrofuran was cooled to -20C under argon atmosphere. Then a 0.5 M solution of potassium bis(trimethylsilyl)amide in toluene (218 mL, 0.109 mol) was added and the mixture was stirred for 1 hr. The 2-benzyloxyethyltriflate (30.9 g, 0.109 mol) was added neat keeping the temperature between -20C and -15C. After the addition was complete, the mixture was allowed to gradually warm to 25C and was stirred for 15 hrs. Dichloromethane (1L) was added to the reaction mixture and the resulting solution was washed with 1L of water, dried over anhydrous sodium sulfate and evaporated under reduced pressure to give crude 1,1,1-tris(benzyloxyethoxymethyl)ethane. This mixture was chromatographed over silica gel using an ethyl acetate/hexane gradient to obtain pure 1,1,1-tris(benzyloxyethoxymethyl) ethane (13.9 g, 0.027 mol, 53%).

A mixture of 1,1,1-tris(benzyloxyethoxymethyl)ethane (13.9 g, 0.027 mol) and palladium hydroxide on carbon, Pd content 20% (2.8 g) in 100 mL of methanol was hydrogenated at 50 psi in a Parr apparatus for 4 hrs. The catalyst was removed by filtration through Celite and the filtrate was evaporated under reduced pressure to give 1,1,1-tris(hydroxyethoxymethyl)ethane (6.7 g, 0.026 mol, 99%).

A solution of 1,1,1-tris(hydroxyethoxymethyl)ethane (6.7 g, 0.026 mol) in 35 mL of anhydrous pyridine was cooled to -10C. Then solid p-toluenesulfonyl chloride (16.6 g, 0.087 mol) was added in portions over 1.5 hrs. The mixture was kept at -10C for 15 hrs. The resulting slurry was partitioned between dichloromethane (100 mL) and water (50 mL) and the layers were separated. The organic layer was washed with water (50 mL), dried over anhydrous sodium sulfate and evaporated to give a crude gum. This

material was purified over silica gel using 1% methanol/dichloromethane as eluant to give 1,1,1-tris(tosylethoxymethyl)ethane (8.9 g, 0.013 mol, 47%).

A slurry of 1,1,1-tris(tosylethoxymethyl)ethane (4.5 g, 6.30 mmol) and potassium phthalimide (3.5 g, 18.90 mmol) in 45 mL of N,N-dimethylacetamide was heated at 80-85C for 15 hrs. The solvent was removed under reduced pressure and the residue was partitioned between dichloromethane and water. The layers were separated and the organic layer was washed with water, dried over anhydrous sodium sulfate and evaporated to give 1,1,1-tris(phthalimidoethoxymethyl)ethane (4.0 g, 6.30 mmol, 100%).

A solution of 1,1,1-tris(phthalimidoethoxymethyl)ethane (4.0 g, 6.30 mmol) in 100 mL of absolute ethanol was heated to 50C. Then a 55% solution of hydrazine hydrate (3.7 mL, 63.00 mmol) was added dropwise and the resulting mixture was heated at 50C for 5 hrs. The reaction was cooled to 25C and the solids were filtered. The filtrate was evaporated to give an oil which was purified over reversed phase packing (C-18) using a methanol/water gradient as eluant to yield 1,1,1-tris(aminoethoxymethyl)ethane (1.3 g, 5.22 mmol, 83%).

To a solution of 1,1,1-tris(aminoethoxymethyl)ethane (1.1 g, 4.40 mmol) in 10 mL of pyridine was added p-toluenesulfonylchloride (2.5 g, 13.2 mmol) in portions over 1 hr. The mixture was stirred at 25_C for 15 hrs. Dichloromethane (100 mL) and water (50 mL) were added to the reaction and the layers were separated. The organic layer was washed three times with 100 mL of 10% hydrochloric acid solution, once with saturated sodium bicarbonate solution (50 mL), dried over anhydrous sodium sulfate and evaporated under reduced pressure. The crude oil was purified by chromatography over silica gel using an ethyl acetate/hexane gradient as eluant to yield 1,1,1-tris(tosylamidoethoxymethyl)ethane (1.2 g, 1.69 mmol, 40%).

A mixture of 1,1,1-tris(tosylethoxymethyl)ethane (1.2 g, 1.69 mmol), 1,1,1-tris(tosylamidoethoxymethyl)ethane (1.2 g, 1.69 mmol) and anhydrous potassium

carbonate,
-325 mesh (0.7 g, 5.1 mmol) in 100 mL of anhydrous N,N-dimethylacetamide was heated at 80-85C under argon atmosphere for 15 hrs.
The solvent was removed under reduced pressure and the residue was partitioned between dichloromethane (100 mL) and water (50 mL). The layers were separated and the organic layer was dried over anhydrous sodium sulfate and evaporated to give a gum. This material was chromatographed over silica gel using an ethyl acetate/hexane gradient as eluant to give 1,11-dimethyl-3,9,13,19, 22, 28-hexaoxa-(N^6, N^{16}, N^{25}-tritosylamido)-6,16,25-triaza-bicyclo[9.9.9]nonacosane (0.34 g, 0.37 mmol, 22%).

A solution of 1,11-dimethyl-3,9,13,19,22,28-hexaoxa-(N^6, N^{16}, N^{25}-tritosylamido)-6,16,25-triaza-bicyclo[9.9.9]nonacosane (0.08 g, 0.09 mmol) in 3 mL of tetrahydrofuran was added to liquid ammonia (10 mL) in a dry ice/
isopropanol bath. Sodium pellets were added as needed to maintain a dark blue color. The bath was removed and the solution was allowed to reflux for 30 min. Then solid ammonium chloride was added to decolorize the solution and the solvents were allowed to evaporate. The residue was triturated with methanol and the solvents were evaporated. The oil was dissolved in water and purified through reversed phase packing (ODS) using a methanol/water gradient as eluant to give 1,11-dimethyl-3,9,13,19,22,28-hexaoxa-6,16,25-triaza-bicyclo[9.9.9]nonacosane (0.01 g, 0.02 mmol, 25%).

A mixture of 1,11-dimethyl-3,9,13,19,22,28-hexaoxa-6,16,25-triaza-bicyclo[9.9.9]nonacosane (10.0 mg, 0.02 mmol), t-butylbromoacetate (12.0 mg, 0.06 mmol), and diisopropylethylamine (8.0 mg, 0.06 mmol) in 2 mL of anhydrous acetonitrile was heated at 50C for 15 hrs. The solvents were removed under reduced pressure and the residue was partitioned between dichloromethane (50 mL) and water (10 mL). The layers were separated and the organic layer was dried over anhydrous sodium sulfate and evaporated to give 1,11-dimethyl-3,9,13, 19,22,28-hexaoxa-[N^6, N^{16}, N^{25}-tri(t-butoxycarbonylmethyl)]-6,16,25-triaza-bicyclo[9.9.9]nonacosane (14.0 mg, 0.02 mmol, 88%).

To a solution of 1,11-dimethyl-3,9,13,19,22,28-hexaoxa-[N^6, N^{16}, N^{25}-tri(t-butoxycarbonylmethyl)]-6,16,25-triaza-bicyclo[9.9.9]nonacosane (14.0 mg, 0.02 mmol) in 2 mL of dichloromethane is added trifluoroacetic acid (0.50 mL). The solution is stirred at 25C for 15 hrs. The solvents are removed under reduced pressure and the residue is dissolved in water. The pH of the aqueous solution is adjusted to 7 with ammonium hydroxide and the solution is chromatographed over reversed phase packing (ODS) using water as eluant to give 1,11-dimethyl-3,9,13,19,22,28-hexaoxa-(N^6, N^{16}, N^{25}-tricarboxymethyl)-6,16,25-triaza-bicyclo[9.9.9]nonacosane (12.0 mg, 0.02 mmol, 100%).

Example 2

Synthesis of 1, 11-dimethyl-3, 9, 13, 19, 22, 28-hexaoxa-(N^6, N^{16}, N^{25}-tricarboxymethyl)-6, 16, 25-triaza-bicyclo[9.9.9]nonacosane, gadolinium(III) complex.

To a solution of 1,11-dimethyl-3,9,13,19,22,28-hexaoxa-(N^6, N^{16}, N^{25}-tricarboxymethyl)-6,16,25-triaza-bicyclo[9.9.9]nonacosane (12.0 mg, 0.02 mmol) in 1 mL of deionized water is added gadolinium oxide (3.6 mg, 0.01 mmol). The mixture is heated at 80C under argon atmosphere for 15 hrs. The resulting solution is filtered and the filtrate is evaporated to give 1,11-dimethyl-3,9,13,19,22,28-hexaoxa-(N^6, N^{16}, N^{25}-tricarboxymethyl)-6,16,25-triaza-bicyclo[9.9.9]nonacosane, gadolinium(III) complex (12.4 mg, 0.02 mmol, 100%).

Example 3

Synthesis of 1-hydroxymethyl-11-carboxy-3,9,13,19,22,28-hexathio-(N^6, N^{16}, N^{25}-trisulfomethyl)-6,16,25-triaza-bicyclo[9.9.9]nonacosane, poly-L-lysine conjugate

To a suspension of pentaerythritol (13.6 g, 0.100 mol) in 10 mL of toluene was added freshly distilled triethyl orthoacetate (16.2 g. 18.3 mL, 0.100 mol) and p-toluenesulfonic acid monohydrate (50 mg). The mixture was slowly heated in an oil bath and ethanol was distilled from the mixture over a period of 12 hrs. After removal of the ethanol, the bath temperature was raised to 125C and toluene was distilled off until the solution was homogeneous (about 30 mL). The residue was sublimed (bulb to bulb, ~130_C, 2.5 mmHg) to give 4-(hydroxymethyl)-1-methyl-2,6,7-trioxabicyclo[2.2.2]octane (15.0 g, 0.093 mol, 93%).

Powdered potassium hydroxide (16.2 g, 0.290 mol) is suspended in 100 mL of dimethyl sulfoxide and the mixture is stirred at 25C for 5 min. Then 4-(hydroxymethyl)-1-methyl-2,6,7-trioxabicyclo[2.2.2]octane (10.0 g, 0.062 mol) is added followed by bromomethyl methyl ether (9.3 g, 6.1 mL, 0.074 mol). The reaction mixture is stirred for 30 min and then diluted with water (1L) and extracted with dichloromethane several times. The combined organic layers are washed with saturated sodium chloride (100 mL), dried over anhydrous sodium sulfate and evaporated under reduced pressure to give 4-(methoxy)-1-methyl-2,6,7-trioxabicyclo[2.2.2]octane. This crude material is dissolved in methanol (100 mL) and stirred with pyridinium p-toluenesulfonate (1.6 g, 6.200 mmol) in water (100 mL) for 30 min. The pH of the solution is adjusted to 7 with solid sodium bicarbonate and the solvents are removed under reduced pressure. The resulting solids are triturated with methanol and the methanol solution is evaporated to give 2-(methoxymethoxy)-1,1,1-tris-(hydroxymethyl)ethane.

A solution of 2-(methoxymethoxy)-1,1,1-(hydroxymethyl)ethane (10.6 g, 0.059 mol) in 50 mL of pyridine is cooled to 0C and p-toluenesulfonyl chloride (37.1 g, 0.195 mol) is

added in portions over 30 min. The solution is kept at 0C for 15 hrs and then partitioned between dichloromethane (200 mL) and water (100 mL). The layers are separated and the organic solution is washed twice with 200 mL of water. The organic layer is dried over anhydrous sodium sulfate and evaporated under reduced pressure to give 2-(methoxymethoxy)-1,1,1-tris-(tosylmethyl)ethane.

A mixture of 2-(methoxymethoxy)-1,1,1-tris-(tosylmethyl)ethane (26.9 g, 0.042 mol) and potassium thioacetate (15.8 g, 0.139 mol) in 100 mL of absolute ethanol is heated at reflux for 8 hrs. The solvents are removed under reduced pressure and the residue is partitioned between dichloromethane (200 mL) and water (100 mL). The organic layer is dried over anhydrous sodium sulfate and evaporated to give 2-(methoxy)-1,1,1-tris-(thioacetylmethyl)ethane. This crude material is dissolved in 100 mL of methanol and stirred with 100 mL of 10% sodium hydroxide solution at 25C for 2 hrs. The solvents are removed under reduced pressure and the solids are triturated with methanol. The combined methanol solutions are evaporated to give 2-(methoxymethoxy)-1,1,1-tris-(thiomethyl)ethane.

A mixture of 2-(methoxymethoxy)-1,1,1-tris-(thiomethyl)ethane (8.2 g, 0.036 mol), potassium carbonate (14.9 g, 0.108 mol) and N-tosylaziridine (21.3 g, 0.108 mol) in 50 mL of N,N-dimethylacetamide is heated at 80C for 15 hrs. Dichloromethane (200 mL) is added and the mixture is washed with water (100 mL). The organic solution is dried over anhydrous sodium sulfate and evaporated under reduced pressure to give 2-(methoxymethoxy)-1,1,1-tris-[[2-(N-tosylamido)ethyl]-thiomethyl]ethane.

To a solution of 4-(hydroxymethyl)-1-methyl-2,6,7-trioxabicyclo[2.2.2]octane (10.0 g, 0.062 mol) in 200 mL of acetone is added Jones reagent (chromium trioxide and sulfuric acid) until an orange color persisted. The excess oxidant is removed by the addition of 2-propanol until a green color is obtained and the solvents are evaporated under reduced pressure. The residue is partitioned between dichloromethane (100 mL) and water (50 mL) and the layers are separated. The organic layer is washed with water, dried over

anhydrous sodium sulfate and evaporated under reduced pressure to give 4-carboxy-1-methyl-2,6,7-trioxabicyclo[2.2.2]octane.

A mixture of 4-carboxy-1-methyl-2,6,7-trioxabicyclo[2.2.2]octane (9.3 g, 0.053 mol) and 1,3-dicyclohexylcarbodiimide (10.9 g, 0.053 mol) in 100 mL of acetonitrile is stirred at 25C for 30 min. Then 2-methyl-2-propanol (3.9 g, 5.0 mL, 0.053 mol) is added and the resulting mixture is stirred for 15 hrs. The solids are filtered and the filtrate is evaporated under reduced pressure to yield 4-(t-butoxycarbonyl)-1-methyl-2,6,7-trioxabicyclo[2.2.2]octane as a crude oil. This material is dissolved in 100 mL of tetrahydrofuran and stirred with 50 mL of 10% hydrochloric acid for 1 hr. The pH of the solution is adjusted to 7 with solid sodium bicarbonate and the solvents are removed under reduced pressure. The solids are triturated with methanol and the combined methanol solutions are evaporated to give t-butyl-tris(hydroxymethyl)acetate.

A solution of t-butyl-tris(hydroxymethyl)acetate (9.9 g, 0.048 mol) in 50 mL of pyridine is cooled to 0C and then portions of p-toluenesulfonyl chloride (30.1 g, 0.158 mol) are added over 1 hr. The mixture is kept at 0C for 15 hrs. Then dichloromethane (200 mL) is added and the solution is washed four times with 100 mL of 10% hydrochloric acid, once with 100 mL of saturated sodium bicarbonate and once with 100 mL of saturated sodium chloride. The organic layer is dried over anhydrous sodium sulfate and evaporated under reduced pressure to give t-butyl-tris(tosylmethyl)acetate.

Sodium hydride (2.6 g, 0.108 mol) is added slowly to a solution of 2-benzyloxyethanethiol (18.1 g, 0.108 mol) in 200 mL of anhydrous tetrahydrofuran. The slurry is stirred at 25C for 30 min. and then a solution of t-butyl-tris(tosylmethyl)acetate (23.7 g, 0.036 mol) in 100 mL of anhydrous tetrahydrofuran is added dropwise. After the addition is complete, the mixture is heated at 80C for 15 hrs. The reaction mixture is cooled to 25C and water (50 mL) is slowly added. Then the solution is extracted several times with dichloromethane and the combined extracts are dried over anhydrous sodium sulfate and evaporated under reduced pressure to give t-butyl-tris[[(2-benzyloxy)ethyl]thiomethyl]acetate.

A slurry of t-butyl-tris[[(2-benzyloxy)ethyl]thiomethyl]acetate (19.0 g, 0.029 mol) and palladium hydroxide on carbon (0.4 g, 20% palladium) in 200 mL of methanol is hydrogenated at 50 psi using a Parr hydrogenation apparatus. After 4 hrs., the catalyst is removed by filtration through Celite and the filtrate is evaporated under reduced pressure to give t-butyl-tris[(2-hydroxyethyl)-thiomethyl]acetate.

A solution of t-butyl-tris[(2-hydroxyethyl)thiomethyl]acetate (11.2 g, 0.029 mol) in 50 mL of pyridine is cooled to 0C and p-toluenesulfonyl chloride (18.3 g, 0.096 mol) is added in portions over 1 hr. The mixture is kept at 0C for 15 hrs. Then dichloromethane (200 mL) is added and the solution is washed four times with 100 mL of 10% hydrochloric acid, once with 100 mL of saturated sodium bicarbonate and once with 100 mL of water. The organic layer is dried over anhydrous sodium sulfate and evaporated to give t-butyl-tris[(2-tosylethyl)-thiomethyl]acetate.

A mixture of t-butyl-tris[(2-tosylethyl)thiomethyl]acetate (10.0 g, 0.013 mol), 2-(methoxymethoxymethyl)-1,1,1-tris[[2-(N-tosylamido)ethyl]thiomethyl]ethane (9.4 g, 0.013 mol) and potassium carbonate (5.4 g, 0.039 mol) in 1L of anhydrous N,N-dimethylacetamide is heated at 80C under argon atmosphere for 15 hrs. The solvents are removed under reduced pressure and the residue is partitioned between dichloromethane (200 mL) and water (100 mL). The layers are separated and the organic layer is dried over anhydrous sodium sulfate and evaporated to give 1-(methoxymethoxymethyl)-11-t-butoxycarbonyl-3,9,13,19,22,28-hexathio-(N^6, N^{16}, N^{25}-tritosylamido)-6,16,25-triaza-bicyclo[9.9.9]nonacosane.

A solution of 1-(methoxymethoxymethyl)-11-t-butoxycarbonyl-3,9,13,19,22,28-hexathio-(N, N, N-tritosylamido)-6,16,25-triaza-bicyclo[9.9.9]-nonacosane (4.5 g, 3.900 mmol) in 20 ml of tetrahydrofuran is added to liquid ammonia (100 mL) in a dry ice/2-propanol bath. Sodium pellets are added as needed to maintain a dark blue color. The bath is removed and the solution is allowed to stir at refluxing ammonia for 30 min. Then solid ammonium chloride is added to decolorize the reaction solution and the solvents are

evaporated. The residue is triturated with methanol and the combined solutions are evaporated to give 1-(methoxymethoxymethyl)-11-t-butoxycarbonyl-3,9,13,19,22,28-hexathio-6,16,25-triaza-bicyclo[9.9.9]nonacosane.

A solution of 1-(methoxymethoxy)methyl-11-t-butoxycarbonyl-3,9,13,19,22,28-hexathio-6,16,25-triaza-bicyclo[9.9.9]nonacosane (2.0 g, 3.12 mmol) in 20 mL of tetrahydrofuran and 20 mL of water is adjusted to pH 10 with 1N sodium hydroxide. Then chloromethylsulfonic acid (1.2 g, 9.36 mmol) is added and the mixture is stirred at 25_C for 15 hrs., keeping the pH between 9 and 10 with 1N sodium hydroxide. The pH of the solution is brought to 12 with 1N sodium hydroxide and the solution was heated at 100_C for 1 hr. The water is evaporated under reduced pressure to give 1-(methoxymethoxymethyl)-11-carboxy-3,9,13,19,22,28-hexathio-(N^6, N^{16}, N^{25}-trisulfomethyl)-6,16,25-triazabicyclo[9.9.9]nonacosane.
A mixture of 1-(methoxymethoxymethyl)-11-carboxy-3,9,13,19,22,28-hexathio-(N^6, N^{16}, N^{25}-trisulfomethyl)-6,16,25-triabicyclo-[9.9.9]nonacosane (1.0g, 1.15mmol), 1,3-dicyclohexylcarbodiimide (0.24g, 1.15mmol) and N-hydroxysuccinimide (0.13g, 1.15mmol) in 20 mL of acetonitrile is stirred at 25C for 15 hrs. The solid is filtered and the filtrate is added in portions to a sodium bicarbonate buffer solution (pH 8) of poly-L-lysine, molecular weight 1000-4000 (0.92g, 0.23mmol). After 15 hrs. at 25C the solution of 1-(methoxymethoxymethyl)-11-carboxy-3,9,13,19,22,28-hexathio-(N^6,N^{16},N^{25}-trisulfomethyl)-6,16,25-triazabicyclo[9.9.9]nonacosane, poly-L-lysine conjugate, is used to make the dysprosium complex of Example 4.

Example 4

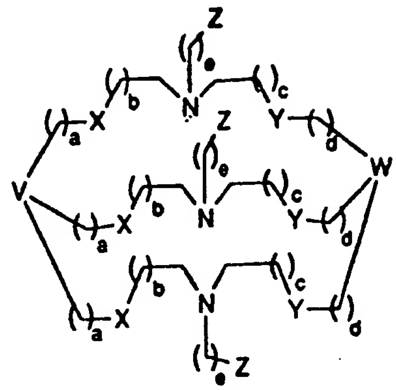
Synthesis of 1-hydroxymethyl-11-carboxy-3,9,13,19,22,28-hexathio-(N^6, N^{16}, N^{25}-trisulfomethyl)-6,16,25-triazabicyclo[9.9.9]nonacosane, poly-L-lysine conjugate, dysprosium(III) complex.

Dysprosium oxide (0.21 g, 0.56 mmol) is added to a solution of 1-(methoxymethoxymethyl)-11-carboxy-3,9,13,19,22,28-hexathio-(N^6, N^{16}, N^{25}-trisulfomethyl)-6,16,25-triazabicyclo-[9.9.9]nonacosane, poly-L-lysine conjugate, prepared in Example 3. The mixture is stirred for 15 hrs and then the pH is adjusted to 4 with 0.1 N hydrochloric acid. After 1 hr the solution is purified through a phosphate buffered Sephadex G-50 gelfiltration column to give 1-hydroxymethyl-11-carboxy-3,9,13,19,22,28-hexathio-(N^6, N^{16}, N^{25}-trisulfomethyl)-6,16,25-triazabicyclo[9.9.9]nonacosane, poly-L-lysine conjugate, dysprosium(III) complex.

Although the invention has been described with respect to specific modifications, the details thereof are not to be construed as limitations, for it will be apparent that various equivalents, changes and modifications may be resorted to without departing from the spirit and scope thereof, and it is understood that such equivalent embodiments are to be included therein.

CLAIMS:

1. A compound of the general formula:



Wherein V is CR_1 or N; W is CR_2 or N; R_1 and R_2 may be the same or different and are hydrogen, C_1-C_8 alkyl, or C_6-C_{10} aryl, optionally R_1 and R_2 may be substituted by one or more hydroxy, C_1-C_8 alkyl, C_1-C_8 hydroxyalkyl, C_1-C_8 alkoxy, C_6-C_{10} aryl, C_6-C_{10} hydroxyaryl, C_6-C_{10} aryloxy, $-CO_2R_3$, $CONR_4R_5$, or $-NR_4R_5$; X and Y may be the same or different and are O, NR_6, S, ~~or~~ CR_7R_8; R_3, R_4, R_5, R_6, R_7 and R_8 may be the same or different and are hydrogen, C_1-C_8 alkyl, C_1-C_8 hydroxyalkyl, or C_1-C_8 alkoxyalkyl; ~~R_4 and R_5 may form a 5 or 6 membered carbocyclic ring optionally containing singularly or in combination nitrogen, oxygen or sulfur;~~ a, b, c, d and e may be the same or different and are zero to about 10; and Z is $-CO_2H$, $-PO_3H_2$, $-SO_3H$, or $-CONHOH$.

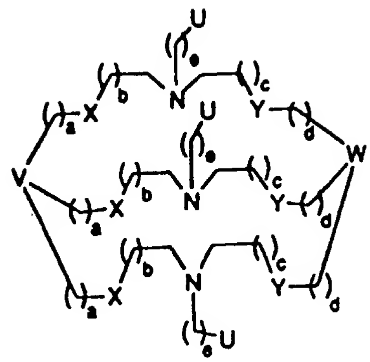
2. The compound of claim 1 wherein V is CR_1; R_1 is CH_3; W is CR_2; R_2 is CH_3; X is O; Y is O; a is 1, b is 1; c is 1; d is 1; e is 1; and z is CO_2H.

3. The compound of claim 1 wherein V is CR_1; R_1 is CH_2OH; W is CR_2; R_2 is $-CH_2CH_2NH$-HSA; X is NR_6; Y is NR_6; R_6 is CH_3; a is 1; d is 1; e is 1; b is 2; c is 2; and Z is CO_2H.

4. The compound of claim 1 wherein V is N; W is N; X is O; Y is O; a is 2; d is 2; b is 1; c is 1; e is 1; and Z is CONHOH.

5. The compound of claim 1 wherein V is CR_1; R_1 is CH_2OH; W is CR_2; R_2 is -CONH-polylysine; X is S; Y is S; a is 1; b is 1; c is 1; d is 1; e is 1; and Z is SO_3H.

6. A compound of the general formula:



Wherein V is CR_1 or N; W is CR_2 or N; R_1 and R_2 may be the same or different and are hydrogen, C_1-C_8 alkyl, or C_6-C_{10} aryl, optionally R_1 and R_2 may be substituted by one or more hydroxy, C_1-C_8 alkyl, C_1-C_8 hydroxyalkyl, C_1-C_8 alkoxy, C_6-C_{10} aryl, C_6-C_{10} hydroxyaryl, C_6-C_{10} aryloxy, $-CO_2R_3$, $CONR_4R_5$, or $-NR_4R_5$; X and Y may be the same or different and are O, NR_6, S, or CR_7R_8. R_3, R_4, R_5, R_6, R_7 and R_8 may be the same or different and are hydrogen, C_1-C_8 alkyl, C_1-C_8 hydroxyalkyl or C_1-C_8 alkoxyalkyl; R_4 and R_5 may form a 5 or 6 membered carbocyclic ring optionally containing singularly or in combination nitrogen, oxygen or sulur; a, b, c, d and e may be the same or different and are zero to about 10; U is $-CO_2M$, $-PO_3HM$, $-SO_3M$ or CONHOM; and M is a metal ion or equivalent and/or a physiologically acceptable cation of an inorganic or organic base.

7. The compound of claim 6 wherein V is CR_1; R_1 is CH_3; W is CR_2; R_2 is CH_3; X is O; Y is O; a is 1; b is 1; c is 1; d is 1; e is 1; U is CO_2M; and M is gadolinium.

8. The compound of claim 6 wherein V is CR_1; R_1 is $-CH_2OH$; W is CR_2; R_2 is $-CH_2CH_2NH(HSA)$; X is NR_6; Y is NR_6; R_6 is CH_3; a is 1; d is 1; e is 1; b is 2; C is 2; U is CO_2M; and M is dysprosium.

9. The compound of claim 6 wherein V is CR_1; R_1 is $-CH_2OH$; W is CR_2; R_2 is $-CH_2CH_2NH(HSA)$; X is NR_6; Y is NR_6; R_6 is CH_3; a is 1; d is 1; e is 1; b is 2; c is 2; U is CO_2M; and M is ytterbium.

10. The compound of claim 6 wherein V is N; W is N; X and Y are O; a and d are 2; b, c and e are 1; U is CONHOM; and M is iron.

11. The compound of claim 6 wherein V and W are N; X and Y are O; a and d are 2; b, c and e are 1; U is CONHOM; and M is ytterbium.

12. The compound of claim 6 wherein V is CR_1; R_1 is CH_2OH; W is CR_2; R_2 is CO-polylysine; X is S; Y is S; a is 1; b is 1; c is 1; d is 1; e is 1; U is SO_3M; and M is gadolinium.

13. The compound of claim 6 wherein V is CR_1; R_1 is CH_3; W is CR_2; R_2 is CH_3; X is O; Y is O; a is 1; b is 1; c is 1; d is 1; e is 1; U is CO_2M; and M is bismuth.

14. The compound of claim 6 wherein V is CR_1; R_1 is $-CH_2OH$; W is CR_2; R_2 is $-CH_2CH_2NH(HSA)$; X is NR_6; Y is NR_6; R_6 is CH_3; a is 1; d is 1; e is 1; b is 2; c is 2; U is CO_2M; and M is holmium.

15. The compound of claim 6 wherein V is N; W is N; X is O; Y is O; a is 2; d is 2; b is 1; c is 1; e is 1; U is CONHOM; and M is dysprosium.

16. The compound of claim 6 wherein V is N; W is N; X is O; Y is O; a is 2; d is 2; b is 1; c is 1; e is 1; U is CONHOM; and M is bismuth.

17. The compound of claim 6 wherein V is CR_1; R_1 is CH_2OH; W is CR_2; R_2 is CO-polylysine; X is S; Y is S; a is 1; b is 1; c is 1; d is 1; e is 1; U is SO_3M; and M is dysprosium.

18. The compound of claim 6 wherein V is CR_1; R_1 is CH_3; W is CR_2; R_2 is methyl; X is O; Y is O; a is 1; b is 1; c is 1; d is 1; e is 1; U is CO_2M; and M is gallium.

19. The compound of claim 6 wherein V is CR_1; R_1 is CH_3; W is CR_2; R_2 is CH_3; X is

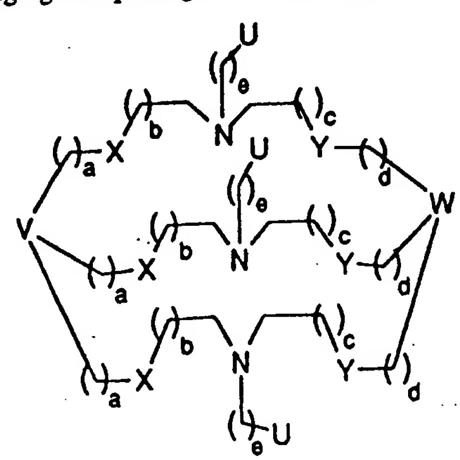
O; Y is O; a is 1; b is 1; c is 1; d is 1; e is 1; U is CO_2M; and M is yttrium.

20. The compound of claim 6 wherein V is CR_1; R_1 is $-CH_2OH$; W is CR_2; R_2 is $-CH_2CH_2NH(HSA)$; X is NR_6; Y is NR_6; R_6 is CH_3; a is 1; d is 1; e is 1; b is 2; c is 2; U is CO_2M; and M is indium.

21. The compound of claim 6 wherein V is CR_1; R_1 is CH_2OH; W is CR_2; R_2 is CO-polylysine; X is S; Y is S; a is 1; b is 1; c is 1; d is 1; e is 1; U is SO_3M; and M is technetium.

22. The compound of claim 6 wherein V is CR1; R1 is CH_2OH; W is CR_2; R_2 is CO-polylysine; X is S; Y is S; a is 1; b is 1; c is 1; d is 1; e is 1; U is SO_3M; and M is rhenium.

23. A method of imaging comprising administering to a patient a compound of the general formula:



Wherein V is CR_1 or N; W is CR_2 or N; R_1 and R_2 may be the same or different and are hydrogen, C_1-C_8 alkyl, or C_6-C_{10} aryl, optionally R_1 and R_2 may be substituted by

one or more hydroxy, C_1-C_8 alkyl, C_1-C_8 hydroxyalkyl, C_1-C_8 alkoxy, C_6-C_{10} aryl, C_6-C_{10} hydroxyaryl, C_6-C_{10} aryloxy, $-CO_2R_3$, $CONR_4R_5$, or $-NR_4R_5$; X and Y may be the same or different and are O, NR_6, S, or CR_7R_8. R_3, R_4, R_5, R_6, R_7 and R_8 may be the same or different and are hydrogen, C_1-C_8 alkyl, C_1-C_8 hydroxyalkyl or C_1-C_8 alkoxyalkyl; R_4 and R_5 may form a 5 or 6 membered carbocyclic ring optionally containing singularly or in combination nitrogen, oxygen or sulur; a, b, c, d and e may be the same or different and are zero to about 10; U is $-CO_2M$, $-PO_3HM$, $-SO_3M$ or CONHOM; and M is a metal ion or equivalent and/or a physiologically acceptable cation of an inorganic or organic base.

24. The method of claim 23 wherein V is CR_1; R_1 is CH_3; W is CR_2; R_2 is CH_3; X is O; Y is O; a is 1; b is 1; c is 1; d is 1; e is 1; U is CO_2M; and M is gadolinium.

25. The method of claim 23 wherein V is CR_1; R_1 is $-CH_2OH$; W is CR_2; R_2 is $-CH_2CH_2NH(HSA)$; X is NR_6; Y is NR_6; R_6 is CH_3; a is 1; d is 1; e is 1; b is 2; C is 2; U is CO_2M; and M is dysprosium.

26. The method of claim 23 wherein V is CR_1; R_1 is $-CH_2OH$; W is CR_2; R_2 is $-CH_2CH_2NH(HSA)$; X is NR_6; Y is NR_6; R_6 is CH_3; a is 1; d is 1; e is 1; b is 2; c is 2; U is CO_2M; and M is ytterbium.

27. The method of claim 23 wherein V is N; W is N; X and Y are O; a and d are 2; b, c and e are 1; U is CONHOM; and M is iron.

28. The method of claim 23 wherein V and W are N; X and Y are O; a and d are 2; b, c and e are 1; U is CONHOM; and M is ytterbium.

29. The method of claim 23 wherein V is CR_1; R_1 is CH_2OH; W is CR_2; R_2 is CO-polylysine; X is S; Y is S; a is 1; b is 1; c is 1; d is 1; e is 1; U is SO_3M; and M is gadolinium.

30. The method of claim 6 wherein V is CR_1; R_1 is CH_3; W is CR_2; R_2 is CH_3; X is O;

Y is O; a is 1; b is 1; c is 1; d is 1; e is 1; U is CO_2M; and M is bismuth.

31. The method of claim 23 wherein V is CR_1; R_1 is $-CH_2OH$; W is CR_2; R_2 is $-CH_2CH_2NH(HSA)$; X is NR_6; Y is NR_6; R_6 is CH_3; a is 1; d is 1; e is 1; b is 2; c is 2; U is CO_2M; and M is holmium.

32. The method of claim 23 wherein V is N; W is N; X is O; Y is O; a is 2; d is 2; b is 1; c is 1; e is 1; U is CONHOM; and M is dysprosium.

33. The method of claim 23 wherein V is N; W is N; X is O; y IS O; a is 2; d is 2; b is 1; c is 1; e is 1; U is CONHOM; and M is bismuth.

34. The method of claim 23 wherein V is CR_1; R_1 is CH_2OH; W is CR_2; R_2 is CO-polylysine; X is S; Y is S; a is 1; b is 1; c is 1; d is 1; e is 1; U is SO_3M; and M is dysprosium.

35. The method of claim 23 wherein V is CR_1; R_1 is CH_3; W is CR_2; R_2 is methyl; X is O; Y is O; a is 1; b is 1; c is 1; d is 1; e is 1; U is CO_2M; and M is gallium.

36. The method of claim 23 wherein V is CR_1; R_1 is CH_3; W is CR_2; R_2 is CH_3; X is O; Y is O; a is 1; b is 1; c is 1; d is 1; e is 1; U is CO_2M; and M is yttrium.

37. The method of claim 23 wherein V is CR_1; R_1 is $-CH_2OH$; W is CR_2; R_2 is $-CH_2CH_2NH(HSA)$; X is NR_6; Y is NR_6; R_6 is CH_3; a is 1; d is 1; e is 1; b is 2; c is 2; U is CO_2M; and M is indium.

38. The method of claim 23 wherein V is CR_1; R_1 is CH_2OH; W is CR_2; R_2 is CO-polylysine; X is S; Y is S; a is 1; b is 1; c is 1; d is 1; e is 1; U is SO_3M; and M is technetium.

39. The method of claim 23 wherein V is CR1; R1 is CH_2OH; W is CR_2; R_2 is CO-polylysine; X is S; Y is S; a is 1; b is 1; c is 1; d is 1; e is 1; U is SO_3M; and M is

rhenium.

PCT

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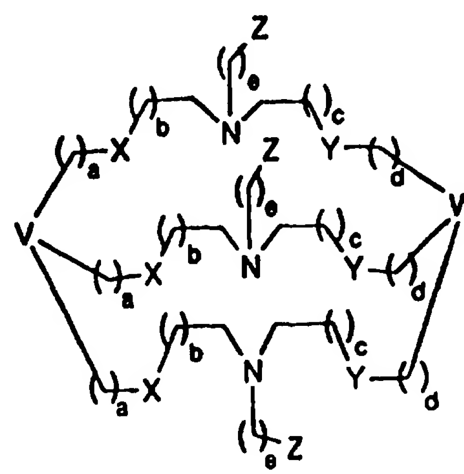
(54) Title: FUNCTIONALIZED AZA-CRYPTAND LIGANDS FOR IMAGING APPLICATIONS

(57) Abstract

The present invention provides new and structurally diverse compositions comprising compounds of general formula (I), wherein V is CR_1 or N; W is CR_2 or N; R_1 and R_2 may be the same or different and are hydrogen, C_1-C_8alkyl, or C_6-C_{10}aryl, optionally R_1 and R_2 may be substituted by one or more hydroxy, C_1-C_8alkyl, C_1-C_8hydroxyalkyl, C_1-C_8alkoxy, C_6-C_{10}aryl, C_6-C_{10}hydroxyaryl, C_6-C_{10}aryloxy, $-CO_2R_3$, $CONR_4R_5$, or $-NR_4R_5$; X and Y may be the same or different and are O, NR_6, S, or CR_7R_8; R_3, R_4, R_5, R_6, R_7 and R_8 may be the same or different and are hydrogen, C_1-C_8alkyl, C_1-C_8hydroxyalkyl, or C_1-C_8alkoxyalkyl; R_4 and R_5 may form a 5 or 6 membered carbocyclic ring optionally containing singularly or in combination nitrogen, oxygen or sulfur; a, b, c, d and e may be the same or different and are zero to about 10; and Z is $-CO_2H$, $-PO_3H_2$, $-SO_3H$, or -CONHOH. Methods for imaging using compositions of the invention are also provided.



(I)

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US94/10998

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :A61B 5/055; A61K 49/04, 51/06; C07D 403/04, 413/04, 419/04
US CL :Please See Extra Sheet.
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/1.65, 1.77, 4, 9; 534/15, 16; 514/183, 186, 431, 450, 836; 436/173; 128/653.4, 654; 540/465, 472

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

STN
chemical structure searched in registry file

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A,P	US, A, 5,322,681 (Klaveness) 21 June 1994, see abstract and claim 1.	1-39
A	US, A, 4,927,923 (Mathis et al.) 22 May 1990, see claim 1.	1-39
A	US, A, 3,531,468 (Park et al.) 29 September 1970, see formula III.	1-39
A,P	US, A, 5,334,371 (Gries et al.) 02 August 1994, see formula I.	1-39
A	US, A, 5,162,525 (Masilamani et al.) 10 November 1992, see claim 1.	1-39

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier document published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search
30 DECEMBER 1994

Date of mailing of the international search report
10 JAN 1995

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Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US94/10999

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☒ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest ☐ The additional search fees were accompanied by the applicant's protest.
☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US94/109 98

A. CLASSIFICATION OF SUBJECT MATTER:
US CL :

424/1.65, 1.77, 4, 9; 534/15, 16; 514/183, 186, 431, 450, 836; 436/173; 128/653.4, 654; 540/465, 472

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING
This ISA found multiple inventions as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.
I. Claims 1, 4, 6, 10, 11, 15, 16, 23, 27, 28, 32, 33, drawn to the compounds of claim 1 wherein both of the variables V & W are nitrogen and X and Y are either oxygen, nitrogen or sulfur; heavy metal complexes comprising said compounds, compositions thereof, and a method of diagnostic imaging using said compounds.

II. Claims 1, 6, and 23, drawn to the compounds of claim 1 wherein both of the variables V & W are nitrogen and X and Y are CR7R8; heavy metal complexes comprising said compounds, compositions thereof, and a method of diagnostic imaging using said compounds.

III. Claims 1, 6, and 23, drawn to the compounds of claim 1 wherein at least one of the variables V & W are CR1 or CR2 and at least one of R4 and R5 join together to form a carbocyclic ring containing at least one nitrogen, oxygen, or sulfur atom; heavy metal complexes comprising said compounds, compositions thereof, and a method of diagnostic imaging using said compounds.

IV. Claims 1, 2, 6, 7, 13, 18, 19, 23, 24, 30, 35, and 36, drawn to the compounds of claim 1 wherein at least one of the variables V & W are CR1 or CR2; R4 and R5, if present, do not form a heterocyclic ring; and X and Y are either oxygen, nitrogen or sulfur; heavy metal complexes comprising said compounds, compositions thereof, and a method of diagnostic imaging using said compounds.

V. Claims 1, 6, and 23, drawn to the compounds of claim 1 wherein at least one of the variables V & W are CR1 or CR2; R4 and R5, if present, do not form a heterocyclic ring; and X and Y are CR7R8; heavy metal complexes comprising said compounds, compositions thereof, and a method of diagnostic imaging using said compounds.
VI. Claim1, 3, 5, 6, 8, 9, 12, 14, 17, 20-23, 25, 26, 29, 31, 34, and 37-39, drawn to the compounds of claim 1 wherein at least one of the variables V & W are CR1 or CR2; R1 or R2 contain a biomolecule; heavy metal complexes comprising said compounds, compositions thereof, and a method of diagnostic imaging using said compounds.

The inventions listed as Groups I-VI do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

First, a reference which would anticipate Group I would not necessarily anticipate or even make obvious the invention(s) of Groups II-VI. Further, the searches of the inventions are not co-extensive, particularly with regard to the literature search required. One skilled in the art could readily practice the invention of Group I without practicing the invention(s) of Groups II-VI. Since each group represents a structurally and chemically independent and distinct group of compounds, and since the special technical feature (i.e. the binding/and bridging groups) appear to be different in each group, a lack of unity requirement appears proper.

BAD ORIGINAL